structure dissociates and a second portion of the said fourth region form a second duplex structure therebetween'...(the phrase) is an extremely complicated phrase and it is unclear what specific structural elements differ between these two duplexes necessarily". Examiner further states that "...for the purpose of the prior art rejections, this claim (cancelled claim 14) will be interpreted such that two duplexes are formed, a first duplex which is denatured and then gives rise to a second duplex that has a restriction site". New claim 87 more accurately describes the mechanism underlying the present methodology and resolves any confusion relating to the structural elements participating in the detection reaction of the present invention. New claim 87 includes the limitations: "...said anchor and said amplifier oligonucleotides including a first region being capable of hybridizing with the target nucleic acid sequence, each of said anchor and said amplifier oligonucleotides further including a second region, said second regions of said anchor and said amplifier oligonucleotides being capable of forming a duplex structure including a nucleic acid cleaving agent recognition sequence following hybridization of said first regions of said anchor and said amplifier oligonucleotides with the target nucleic acid sequence..." (emphasis added).

Applicant wishes to point out that no reference is made to formation of "...a first duplex which is denatured and then gives risc to a second duplex that has a restriction sitc", as in the cancelled claim 14, rather that the sole "duplex structure" refcrrcd to is that formed by the second regions of the anchor and amplifier oligonucleotides "...following hybridization of said first regions of said anchor and amplifier oligonucleotides with the target nucleic acid sequence".

In addition, in order to further clarify the role of each oligonucleotide used by the claimed method and to avoid possible confusion, Applicant has chosen to rename the first oligonucleotide and the second oligonucleotide

recited in the claims as "amplifier oligonucleotide" and "anchor oligonucleotide", respectively. Support for such terminology can be found throughout the instant application, see for example page 42, lines 35-37.

In view of the cancellation of claims 14-26, and their replacement with clearer and more accurate new claims 87-92, as described hereinabove, Applicant believes to have overcome the 35 U.S.C. § 112, second paragraph rejections.

***35 U.S.C. § 102(b) Rejections – Hogan et al (U.S. Pat. 5,451,503)***

The Examiner has rejected claims 41–49, 71–78 and 86 under 35 U.S.C. § 102(b) as being anticipated by Harrison et al. The Examiner's rejections are respectfully traversed. Claims 41–49, 71–78 and 86 have now been cancelled rendering moot the Examiners rejection with respect to these claims. New claims 87-92 have now been added.

The Examiner points out that the "Hogan teaches a method for detecting the presence or the absence of a target nucleic acid sequence in a sample (column 6 and column 35, claim 1) comprising ... 'an assembly of nucleotides where there is a first and second regions which are target specific and third and fourth regions which...hybridize to form a duplex which contains an endonuclease cleavage site...upon hybridization to the target sequence'... 'adding a cleaving agent'...and '...monitoring the presence or absence of cleavage products'".

The Examiner further states that Hogan "...expressly teaches the instance where cleavage of the arm regions reduces the stability of the complex...", "...expressly teaches the use of modified nucleotides in the probes...", "...expressly teaches that this dissociation can enable a second assembly to hybridize with the target sequence", "...teaches the use of two regions of the same polynucleotide, and multiplexing this assay to detect

multiple targets", "...teaches the use of a probe which is self annealing", "...teaches the use of a variety of labels...the use of S1 nuclease detection...and the use of radioactive moieties in detection." The Examiner's statements are respectfully traversed.

Applicant has chosen to pursue the preferred embodiment of the present invention and as such has chosen to replace claims 1-86 with new claims 87-92. It is Applicant's strong opinion that new claims 87-92 which describe a method which utilize preferred oligonucleotide configuration of the present invention are not anticipated by the various oligonucleotide assembly configurations described by Hogan et al.

The method recited in new claims 87-92 includes the limitations of previous claims 41–49, 71–78 and 86. These limitations have now been rewritten in order to more accurately reflect what Applicant considers the claimed invention and to more accurately identify the oligonucleotide features which distinct the claimed invention from the teachings of Hogan et al.

As mentioned above, in order to further clarify the role of each oligonucleotide used by the claimed oligonucleotide system and to avoid possible confusion, Applicant has chosen to rename the first oligonucleotide and the second oligonucleotide recited in the claims as "amplifier oligonucleotide" and "anchor oligonucleotide", respectively. Support for such terminology can be found throughout the instant application, see for example page 42, lines 35-37.

Applicant strongly believes that although Hogan et al. describe several oligonucleotide assembly configurations useful for the detection of a target nucleic acid sequence and methods of utilizing same, such configurations and methods neither anticipate nor do they render obvious the target sequence detection method described in new claims 87-92 of the instant application.

Although Hogan et al, in one embodiment (example 8, column 21) of the

referenced prior art disclose the cleavage of "...an active restriction enzyme site removed from the target strand, ...the cleavage site designed to be close to the base of the arm region duplex" such that "...upon cleavage the arm regions are ...removed from the 3-way branched...structure, reducing the stability of the overall structure", this mechanism of detection of hybridization between probes and target sequences is dependent on the melting of the probes from the target, requiring repetition of the entire hybridization and cleavage process for amplification of detection signal.

In sharp distinction from Hogan et al., the oligonucleotides of the method of new claims 87-92 are designed with the intention of decreasing the orders of the hybridization and dissociation (following cleavage) reactions between the oligonucleotide probes and the target nucleic acid sequence, since such a decrease in reaction order results in an increase in signal amplification.

To decrease the reaction order, the inventors have designed one oligonucleotide ("anchor") such that it stably hybridizes with the target nucleic acid sequence under the reaction conditions used. Such stable hybridization between the anchor oligonucleotide and target can be provided, for example, by a target complementary sequence (first region of the anchor oligonucleotide) of a Tm substantially higher that the reaction temperature.

The second oligonucleotide of the system (the "amplifier") is designed such that it hybridizes with the target nucleic acid sequence substantially only in the presence of a hybridized anchor oligonucleotide thus increasing hybridization efficiency and decreasing the likelihood of non-specific hybridization. Following hybridization of the amplifier oligonucleotide with the target-anchor, a nucleic acid cleavage site is formed which is nuclease-cleavable only in the amplifier sequence ("second region" of the amplifier oligonucleotide). Whereas the oligonucleotide system disclosed by Hogan et al forms a nucleic acid cleavage site comprising two discontinuous

complementary strands, the "duplex structure" including a nucleic acid cleavage site formed by the oligonucleotides of the method of the instant application is a result of the presence of a looped structure (see FIG. 1 of the instant application) of the amplifier, and the short complementary sequence of the anchor oligonucleotide. This constitutes a uniquely stable structure when hybridized, since the loop and nick inherent in the (amplifier-anchor) duplex structure (see FIG. 1 of the instant application) produce stabilizing stem-stacking forces.

Applicant wishes to point out that, in contrast to the nucleic acid cleavage site described by Hogan et al, the cleavage site formed by hybridization of the anchor and amplifier oligonucleotides utilized by the method of the present invention, while double stranded in it's recognition sequence, is cleaved only on one strand since the complementary strand is nicked (see FIG. 1 of the instant application). The presence of a single-stranded cleavage site greatly enhances the efficiency of nuclease digestion, enhancing signal generation and sensitivity of detection of the target sequence.

Such cleavage of the duplex structure destabilizes the amplifier oligonucleotide and causes its release from the target, while the anchor oligonucleotide remains hybridized to the target ("oligonucleotide-target nucleic acid sequence hybrid") and as such is recycled along with it and used in each of the subsequent self-cycling steps of the reaction.

The above-described features of the oligonucleotides of the present invention are clearly recited in claim 87:

"said anchor and said amplifier oligonucleotides are selected such that when hybridized with the target nucleic acid sequence in a presence of a nucleic acid cleaving agent recognizing said nucleic acid cleaving agent recognition sequence, only said amplifier oligonucleotide is cleaved by said nucleic acid cleaving agent, wherein said cleavage of said amplifier oligonucleotide leads to

dissociation of said amplifier oligonucleotide from the target nucleic acid sequence while said anchor oligonucleotide remains hybridized to the target nucleic acid sequence to form a stabilized anchor oligonucleotide-target nucleic acid sequence hybrid thereby allowing a second and uncleaved amplifier oligonucleotide to hybridize with said anchor oligonucleotide-target nucleic acid sequence hybrid thus enabling recycling of said anchor oligonucleotide-target nucleic acid sequence hybrid with respect to said amplifier oligonucleotide" (emphasis added).

Upon careful review of U.S. Pat. No. 5,451,503, Applicant is of an opinion that the method of detection of the present invention (see Figure 1 of the instant application for a schematic illustration) is neither described nor is it suggested by Hogan et al.

New claim 87 of the instant application describes a method using a stable target-anchor oligonucleotide hybrid in which the anchor oligonucleotide is not cleaved nor is it modified during the reaction and which does not dissociate from the target sequence, enabling self-recycling of the "anchor oligonucleotide-target nucleic acid sequence hybrid", thereby decreasing the order of the reactions.

In sharp contrast, in the oligonucleotide assemblies described by Hogan et al., the target nucleic acid sequence recycles with respect to both oligonucleotides used, since both oligonucleotides dissociate from the target following cleavage and as such both oligonucleotides need to be constantly replaced in each of the subsequent reaction-cycling steps.

A reduction of reaction order due to stable hybridization between the anchor oligonucleotide and the target is characteristic both for the 3-hybrid formation (target-anchor-amplifier) reaction and also for the subsequent cleavage and dissociation reaction.

In addition to decreasing reaction order, the use of an anchor

oligonucleotide enables minimizing the quantity (copy number) of such an oligonucleotide used in the reaction, thus minimizing or eliminating background signals associated with non-specific hybridization resultant from the presence of larger oligonucleotide quantities. Furthermore, hybridization of the anchor oligonucleotide to the target provides the target sequence with additional chain length and volume, thereby increasing the available interface area for the subsequent hybridization with the amplifier oligonucleotide. Such an increase in hybridization interface area results in both increased efficiency and specificity of hybridization with the amplifier.

The oligonucleotide configuration of the present invention was compared to prior art oligonucleotide designs (e.g., Hogan et al.) as far as specific cleavage efficiency and signal generation. The results are presented in Examples 4-5 of the instant application. As illustrated therein, it is abundantly clear that the novel and unique features characterizing the oligonucleotide system of the present invention enable such a system to more efficiently detect target sequences than prior art oligonucleotide assemblies including that described by Hogan et al. The oligonucleotide system of the present invention exhibits a several fold increase in both cleavage efficiency and signal generation over prior art oligonucleotide assemblies, thus enabling the method of the present invention to detect target concentrations which were previously undetectable by prior art methodology.

Thus, in view of the arguments presented above it is Applicant's strong opinion that Hogan et al does not anticipate, nor render obvious the invention claimed in new claims 87-92.

***35 U.S.C. § 102(b) Rejections – Lizardi et al (U.S. Pat. 5,118,801)***

The Examiner has also rejected claims 14-19, 41-44, 46, 71-73, 75, 77 and 86 under 35 U.S.C. § 102(b) as being anticipated by Lizardi et al. (U.S. Pat.

No. 5,118,801). The Examiner's rejections are respectfully traversed. Claims 14-19, 41-44, 46, 71-73, 75, 77 and 86 have now been cancelled, thus rendering moot the Examiner's rejections with respect to these claims. New claims 87-92 have now been added.

The Examiner has stated that Lizardi et al teach " a method for detecting the presence or absence of a target nucleic acid sequence ...comprising... an assembly of oligonucleotides... where there is a first and second regions which are target specific... and third and fourth regions...which hybridize to form a duplex...where, upon hybridization of the first and second target regions with the target...sequence, said first duplex structure dissociates, and a new duplex is formed between a second oligonucleotide... and one of the dissociated duplex strands."

Applicant is of the strong opinion that the "...probe containing a molecular switch..." as disclosed by Lizardi et al, does not anticipate or render obvious the methods taught by the invention claimed in new claims 87-92.

Lizardi et al disclose nucleic acid hybridization probes comprising three essential ingredients: "... a probe sequence and complementary switch sequences on both sides of the probe"(column 6, lines 40-43, also FIG. 1), designed such that "...when a nucleic acid double helix is formed between a relatively short probe sequence and a target sequence, the ends of the double helix are necessarily located at a distance from each other..."(column 5, lines 24-27). This displacement of the "complementary switch sequences", resulting in detectable signal generation, is essential to the functioning of the probes of claims 1- 65 (columns 15-20) recited in Lizardi et al. However, no mention is made of separate "anchor" and "amplifier" oligonucleotides, designed such that only when *brought into approximation* by hybridization to the target sequence, interact to form a nucleic acid cleavage site. Rather, it is precisely the distance imposed upon the "complementary switch sequences" by hybridization to the

target sequence that activates the various detection methods disclosed by Lizardi et al. Further, the double helix formed by hybridization of the probes to the target sequence as disclosed by Lizardi et al is in no way destabilized by cleavage of nuclease recognition sites, and as such requires remelting and repetition of the entire recognition process for detection of the target nucleic acid sequence.

In sharp contrast to the probes disclosed by Lizardi et al, the "amplifier" and "anchor" oligonucleotides of the method of the present invention, as recited in new claims 87 are "capable of forming a duplex structure including a nucleic acid cleavage agent recognition sequence following hybridization of said...anchor and...amplifier oligonucleotides with the target nucleic acid sequence", further designed "...such that when hybridized with the target nucleic acid sequence in a presence of a nucleic acid cleaving agent recognizing said nucleic acid cleaving agent recognition sequence, only said amplifier oligonucleotide is cleaved by said nucleic acid cleaving agent, wherein said cleavage of said amplifier oligonucleotide leads to dissociation of said amplifier oligonucleotide from the target nucleic acid sequence while said anchor oligonucleotide remains hybridized to the target nucleic acid sequence to form a stabilized anchor oligonucleotide-target nucleic acid sequence hybrid thereby allowing a second and uncleaved amplifier oligonucleotide to hybridize with said anchor oligonucleotide-target nucleic acid sequence hybrid thus enabling recycling of said anchor oligonucleotide-target nucleic acid sequence hybrid with respect to said amplifier oligonucleotide" (emphasis added).

Thus, in sharp contrast to the teachings of Lizardi et al, in the method of the present invention, *approximation* of the signal-generating portions of the "amplifier" and "anchor" oligonucleotides, through hybridization with the target sequences, is essential for detection of the target sequence and recycling, since such approximation leads to cleavage which destabilizes the "amplifier"

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